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Progress Report

RESISTANCE OF PINES TO BARK BEETLES

Studies on Toxicity of Resins

1961

By Richard H. Smith, Entomologist

NOT FOR PUBLICATION

U. S. DEPARTMENT OF AGRICULTURE, FOREST SERVICE
PACIFIC SOUTHWEST FOREST AND RANGE EXPERIMENT STATION

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U. S. DEPARTMENT OF AGRICULTURE - FOREST SERVICE
PACIFIC SOUTHWEST FOREST AND RANGE EXPERIMENT STATION
Division of Forest Insect Research

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SUMMARY

The major objectives, the beetles, the resins and resin derivatives, and the general procedures of the 1961 host-resistance research were similar to those of previous seasons. All field work was done at the Institute of Forest Genetics at Placerville.

Beetles were reared in an insectary at the Institute from naturally infested hosts gathered from the Central Sierra. Daily application of a water spray to Dendroctonus monticolae brood logs in the insectary seemed to improve the conditions for development and emergence. This procedure did not improve the conditions for D. jeffreyi.

Two procedures were used to determine the effect of resin vapors on adult bark beetles. One was the vapor toxicity procedure used in previous work to determine a lethal affect; the other measured the effect of resin vapor on the feeding capabilities of the beetle as expressed by frass production.

Results with Dendroctonus monticolae were as follows: Ponderosa pine resin vapor was toxic in some tests which sometimes were associated with high vapor saturation. There were indications that a high rate of natural mortality might affect the results of vapor toxicity tests also. Ponderosa pine resin vapor caused little or no reduction in frass production. The vapors of the supernatant liquid and of the low temperature distillate of ponderosa pine caused no reduction in frass production.

Resin vapor of three other hosts, Coulter, sugar, and lodgepole pines, caused no significant increase in mortality or decrease in frass production.

Jeffrey pine resin vapor caused a highly significant reduction in frass production which is largely attributed to the rapid rate of mortality.

Resin vapors of host x nonhost hybrids (Jeffrey x ponderosa and Jeffrey x Coulter) did not increase beetle mortality but did inhibit feeding. Thus, the feeding tests were able to demonstrate a deleterious nonlethal effect of resin vapor.

Resin vapors of the closed cone pines and their hybrid (Monterey, knobcone, and knobcone x Monterey), all of which are nonhosts, usually caused an increase in beetle mortality but had a variable effect on feeding.

Results with Dendroctonus brevicomis were as follows: Feeding behavior of the beetles was not adversely affected by ponderosa pine resin vapor; however, the vapor may sometimes be toxic to the beetles because of variations in either the beetle or the resin. The supernatant liquid of ponderosa pine resin performed much like fresh resin in vapor toxicity tests. The low temperature distillate caused a significant increase in mortality; there was a significant difference between the mortality caused by two samples of evaporate obtained at different times of the season.

Coulter pine resin vapor was nontoxic and did not inhibit frass production. Resin vapors of nonhosts knobcone and lodgepole pines usually produced a significant increase in beetle mortality.

Resin vapors of Jeffrey x ponderosa and Jeffrey x Coulter pines had a sublethal effect on the beetles which apparently inhibited their ability to feed. Effects of the vapors on beetle survival were variable.

The closed-cone pines (knobcone, Monterey and the knobcone x Monterey hybrid) usually caused a significant increase in mortality in vapor toxicity tests.

Soft pine resin vapors did not adversely affect beetle survival or feeding activities.

Some of the variable results obtained in feeding tests with both beetles may be caused by the absorptive action of the frass and bark, preventing saturation of the resin vapors in the chamber.

Resin volatility and vapor saturation in 1961 varied only slightly from that of 1960. The greatest difference was in the vapor saturation of ponderosa and Jeffrey pine resins.

INTRODUCTION

Research on the resistance of pines to bark beetles was conducted along much the same lines in 1961 as in 1960. The general objective of this work is to identify host factors that affect the success of bark beetle attacks, and to determine how they operate. The research program in 1961 was concerned primarily with the toxicity of pine resins to adult beetles, an aspect of resistance that has been under study for 4 years. The test materials have been resins from various pine species and hybrids; the test insects have been three species of Dendroctonus. Progress of the research up to 1961 is summarized in some detail in last year's report.[1/]

1/ Smith, R. H. 1961. Progress report, resistance of pines to bark beetles, studies on toxicity of resins, 1960. U. S. Forest Serv., Pac. Southwest Forest and Range Expt. Sta. 51 pp., illus. (processed).

Leads suggested and questions presented by the previous studies were pursued in this year's work. One lead, which was indicated by variable results obtained with the vapor toxicity procedure with hybrid resins, was the possibility of resin vapors having a nonlethal deleterious effect on the beetles, particularly resin of a host x nonhost hybrid. This suggested the need for a refinement in basic technique which would demonstrate such an effect. It suggested also the possibility that such a refinement might provide an answer to why toxicity results were different with nonhost soft pine resins than with nonhost hard pine resins.

Seasonal variation in ponderosa pine resin was a second lead indicated by previous work. The periodic extraction of resin from one tree for use in a common vapor toxicity test was the approach chosen to determine whether or not such variations exist. Since fresh resin does not hold well, a Hickman still was used to obtain a low temperature distillate--a clear liquid that does not undergo the obvious physical changes of aging fresh resin.

A third lead suggested by the results of resin vapor toxicity tests was a need for additional evidence to support the hypothesis that bark beetles can tolerate saturated resin vapors of host pines but cannot tolerate those of nonhost pines. Lodgepole pine was added to the list of test resins, and the use of resin from knobcone pine, the knobcone x Monterey hybrid, and the Jeffrey x Coulter hybrid was enlarged.

The subject of bark beetle response was a fourth lead, and one that still presents many important questions with respect to the resistance of pines to bark beetle attack. Attempts to investigate this subject have been frustrated in the past by the lack of reliable techniques. This season's efforts along these lines were no exception. Studies of bark beetle response were quite fruitless because of the lack of adequate procedures.[2]

2/ Since these studies were conducted, Vité and Gara have reported a technique which is said to be effective (see Vité, J. P., and Gara, R. T. 1961. A field method for observation on olfactory responses of bark beetles (Scolytidae) to volatile materials. Contrib. Boyce Thompson Inst. 21(3): 175-182, illus.). It involves the use of activated charcoal to trap odors from fresh bark beetle attacks, followed by the use of the "enriched" charcoal to attract bark beetles under forest conditions.

The 1961 field work was carried out at the Institute of Forest Genetics, Placerville, California. The Institute's facilities were made available to the work, and its personnel gave assistance when the occasion so indicated. Melvin D. Sage ably assisted in the collection of the field data.

PROCEDURES

COLLECTING AND HANDLING BEETLES

Brood material was collected and handled in much the same way as in 1960. About four cords of *D. monticolae* brood logs, 18-inch length, were obtained from a ponderosa pine stand at Crystal Bay, Nevada, in mid-June. Brood development at that time ranged from fully grown larvae to callow adults. Half of the material was put directly into the insectary; the other half was held in the 35° F. coldroom for about 1 month and then moved to the insectary. An additional half cord of lodgepole pine brood material was cut near Camp Richardson. Emergence of beetles from this material was very poor, and only the brood emerging from ponderosa pine was used in the tests. Two and one-half cords of *D. jeffreyi* brood material was cut from Jeffrey pine near Fallen Leaf Lake in mid-June and put directly into the insectary.

D. brevicomis brood material was obtained throughout the summer by stripping bark from large infested ponderosa pines in the Kyburz-Silver Fork area. Bark was removed when the brood had reached the late larval or pupal stage. Part of the bark was put in the insectary while the rest was put in the coldroom and moved to the insectary as needed.

An effort was made to improve conditions for the development and emergence of *D. monticolae* and *D. jeffreyi* by applying a fine water spray to the bolts in the insectary once or twice a day. Last season's work indicated that rapid drying out of the infested bolts in the insectary may have reduced the ability of the beetle to develop and emerge properly. The watering apparently increased the moisture content and lowered the temperature of the brood material. The change in moisture seemed to improve noticeably the *D. monticolae* emergence, while the decreased temperature seemed to reduce the peaking of the emergence and spread it out over a longer period of time. No such improvement was noted with *D. jeffreyi*. Thus, as in 1960, the emergence of this species was far below expectations; consequently, testing with this beetle had to be drastically reduced. Furthermore, the results of such tests as were conducted with *D. jeffreyi* may be questionable because the behavior and reactions of the beetle could not be considered normal or typical.

Beetles were collected twice daily at about 8 a.m. and 8 p.m. in individual #000 gelatin capsules and were held at 35° F., usually for 24 to 48 hours, until used in tests. Therefore, the average beetle had been emerged for 6 hours and held for 1 to 2 days at 35° F. prior to use. Beetles were equitably allocated to all replicates in a test according to size, by ocular estimate. Replicates were randomly assigned to treatments. The row-block order established by this assignment was maintained for the course of the test and the subsequent statistical analysis.

COLLECTING RESIN

The procedures used to obtain and handle resin and other test materials and the trees which were the sources of these materials were the same as those used in 1960. In addition, lodgepole pine (*Pinus murrayana*) resin and a distillate of ponderosa pine resin were also included in the season's tests. The lodgepole pine resin was obtained from a large mature tree near Strawberry on Route 50; the ponderosa resin distillate was obtained by processing fresh resin of the same tree used in other tests in a Hickman still at specified controlled temperatures, usually 40° C.

In processing resin with a Hickman still (figure 1), fresh resin was injected with a hypodermic syringe into the chamber of the still via the upper arm "a." A low constant temperature was applied to the bottom "b" of the still and ice was used to cool the concave ceiling "c." As the vapors condensed on the ceiling, they concentrated into a drop on the downward projecting nipple "d," and when the drop became large enough, it fell into the cup and was trapped in a receptacle at the end of the lower arm "e."

Fresh resin was collected with a microtapping device, and for test purposes samples of the resin were apportioned with a 10 cc. pipette into 3 cc. vials. One sample, usually weighing 200 to 300 mg., was placed in each fumigation chamber. The latter consisted of an air-tight 150 cc. screw cap jar in which beetles in fumigation cells or feeding tubes were also placed. Samples of each test material were serially weighed to obtain the weight of the vapor produced in the chamber during the treatment period. The sample weights were considered representative of the material in the test. After the test was completed, the resin samples were subjected to additional processing to obtain data on percent volatility at various conditions of time and temperature in an open atmosphere.

CONDUCTING VAPOR TOXICITY TESTS

Vapor toxicity was determined as in previous studies by confining the beetles to individual cells within the fumigation chamber (150 cc. jar) with substance to be tested. Each test was replicated. A replicate of *D. brevicomis* consisted of 12 beetles in one jar; a replicate of *D. monticolae* or *D. jeffreyi* consisted of 10 beetles in two jars. Beetles were kept in the chamber for a specified length of time, usually 5 and 7 days, respectively, for *D. brevicomis* or *D. jeffreyi* and *D. monticolae*. At the end of the treatment period the jars were uncapped and a count made of the living and dead beetles in each replicate. The criterion for a dead beetle was the lack of movement when agitated. Beetles were held in a nonresinous atmosphere for observation on subsequent mortality counts after the treatment period.

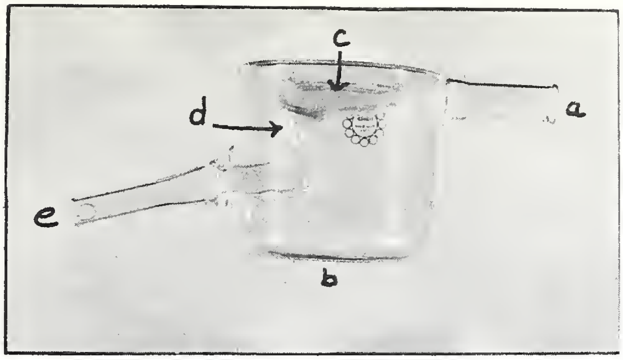

Figure 1.--Hickman still, 3 inches in diameter.

a. Upper inlet arm

b. Bottom

c. Concave ceiling

d. Nipple

e. Lower outlet arm

CONDUCTING FEEDING BEHAVIOR TESTS

The procedure devised for testing the effect of resin vapor on feeding behavior was similar to that used for vapor toxicity, except that the cells in which the individual beetles were confined were replaced with feeding tubes. Each feeding tube consisted of a 3-inch length of glass tubing, 5 to 7 mm. in diameter, containing plugs of ponderosa pine middle bark. The bark plugs were obtained from the rather indefinite zone between the phloem and outer corky bark. Tangential strips of bark, approximately one-quarter inch thick, were sawn from thick bark plates of ponderosa pine obtained from the base of large-diameter trees or stumps. Bark plates from trees of this size are almost flat. Plugs were cut from these tangential strips with a multidiameter leather punch (figure 2). These plugs were, therefore, small cylinders about one-quarter inch long and from 5 to 7 mm. in diameter.

A small amount of distilled water was added to the plugs to raise their moisture content above fiber saturation and to facilitate handling. The plugs were then forced into one end of a 3-inch glass tube so that there was one-quarter inch space between the last plug and the near end of the tube (figure 3). Three plugs were used for *D. monticolae* and *D. jeffreyi*, and two for *D. brevicomis*. A small

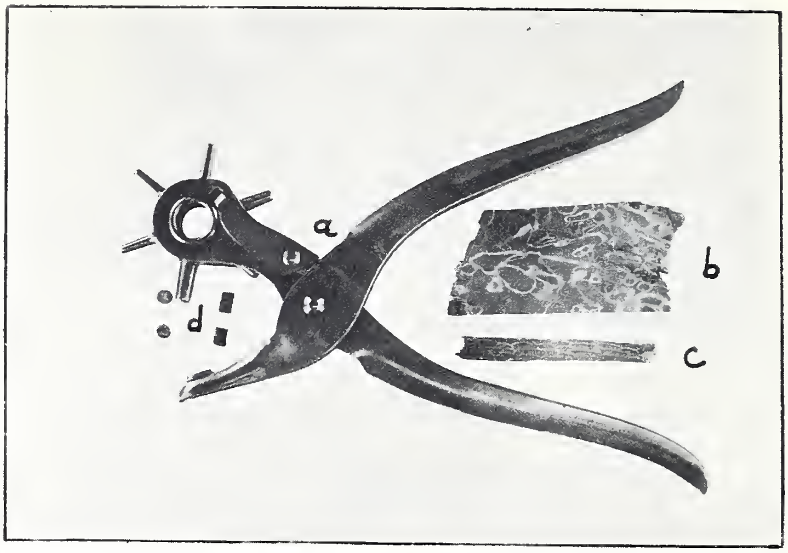

Figure 2.--Leather punch (a) used to make plugs (d) from tangential strip of bark (b), shown in cross section (c).

piece of lumite screening was placed in the quarter-inch space between the end of the tube and the plugs to fit the curvature of the inside of the glass tube for about one-third of the circumference. This afforded the beetle a footing for starting to bore into the plugs. One beetle was installed in each feeding tube by placing it in the quarter-inch space, and closing the near end with a disk of lumite mesh.

Replicates of the assembled feeding tubes were placed in the fumigation chamber along with a resin sample as in the vapor toxicity test. A replicate consisted of ten feeding tubes in one chamber for *D. brevicomis* and ten feeding tubes in two chambers for *D. monticolae* or *D. jeffreyi*. The assembled chambers were placed on a 10° slant with the beetle at the bottom. Thus, the beetles had to bore slightly upward into the plugs.

At the end of the treatment period, the test jars were disassembled, and data collected for each feeding tube on (a) whether the beetle was living or dead based on movement when agitated; (b) whether the beetle had attempted to bore into the plugs; (c) the number of plugs into which the beetle had bored; and (d) the quantity of frass produced. All data were totaled by replicates, and the air-dried weight of the frass was determined. In most cases the volume of frass was also measured.

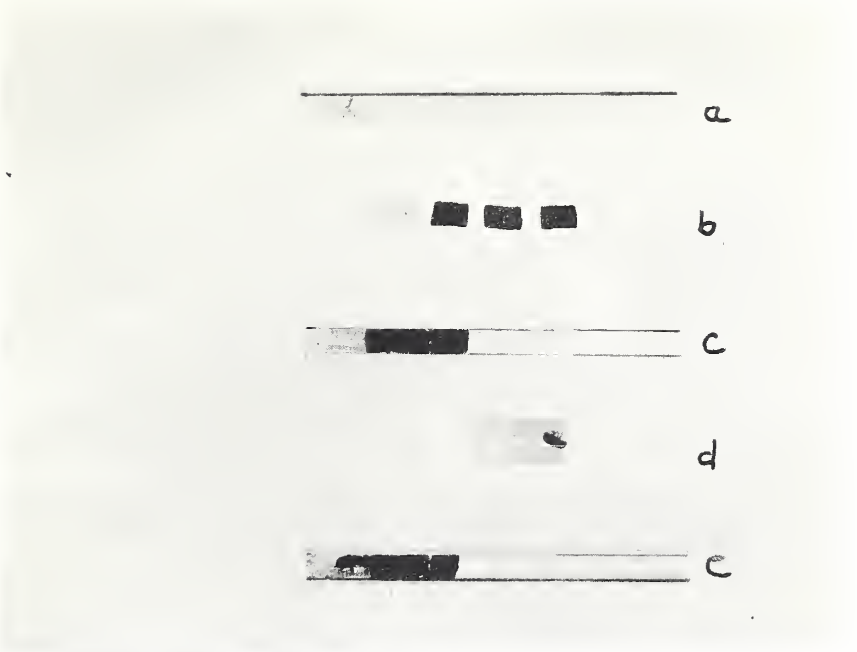

Figure 3.--Feeding tube.

a. 3-inch glass tube

b. 3 bark plugs and lumite mesh

c. a. plus b.

d. Lumite mesh disc and bark beetle

e. c. plus d.

ANALYSIS AND PRESENTATION OF DATA

Results of the vapor toxicity experiments were analyzed by making an F-test of the mortality data for each replicate. Duncan's multiple-range testing was used whenever an F-value was obtained which was greater than "F" at 95 percent. The multiple-range testing was used to discriminate treatments which differed at the 95 percent level.

Data for the feeding-behavior tests were analyzed by applying the F-test to replicate weight and/or volume of frass. Weight was considered the most accurate measure and is the one given within the discussion. A comparison of the weight and volume F-values is given within the report. Duncan's multiple range testing was used as in the vapor toxicity tests. The remaining types of data collected in these tests were not found suitable or significant for analysis. There was little variation among treatments in the number of beetles which attempted to feed. Thus, none of the resin vapors appeared to prevent the action of feeding. Data on number of plugs bored and volume of frass are not given because weight of frass is a more accurate measure of feeding activity; the volume of frass and number of plugs bored are easier to obtain and were used as quick measures of test results.

The basic data and calculations from which the summary tables in the text were compiled are on file in Berkeley.

RESULTS AND DISCUSSION

DENDROCTONUS MONTICOLAE

Vapor Toxicity Tests

Seven vapor toxicity tests were made to recheck some of last season's work with *Dendroctonus monticolae* and the saturated resin vapors of certain pines, and to test other pine resins not used previously (table 1). Each test included an untreated control and ponderosa pine resin.

Ponderosa resin vapor caused no significant increase in mortality in four tests; in the other three there was a significant increase. These results are quite different from last year's in which ponderosa resin vapor never caused a significant mortality increase. In the 1960 tests the difference in mortality between the control and ponderosa resin was always quite small; whereas in 1961 there was an appreciably greater mortality in tests with resin, although in some tests the difference was not significant. Both the rate of natural mortality of *D. monticolae* and the vapor saturation of ponderosa pine in this year's tests were higher than last year's. The natural mortality at 7 days increased from about 25 to over 30 percent, while the vapor saturation increased from about 2.5 to 3.5 mg. Whether these two factors individually or in combination could cause the different results in 1961 cannot be answered on the basis of these tests.

Table 1.--Mortality of D. monticolae in vapor toxicity tests using saturated vapors of indicated resins for 7 days[1] at 73° ± 2° F.

Resin	Vapor saturation average	Test[2] a	b	c	d	e	f	g
	Mg.	Percent[3]						
Control	0.0	37	38	45	37	25	23	27
Ponderosa #1	3.8	55*	55	57	57	45	45*	40*
Ponderosa #2	3.3	--	--	--	--	--	53*	--
Ponderosa supernatant	3.4	--	--	--	--	62*	47*	43*
Coulter	2.3	--	--	--	48	--	--	--
Lodgepole	2.5	43	55	43	--	--	--	--
Knobcone	6.0	68*	65*	63	--	--	--	--
Monterey	4.6	--	--	63	--	--	--	--
Knobcone x Monterey	5.0	--	--	60	--	--	--	--
Jeffrey x Coulter	11.0	20**	30	--	--	--	--	--
Jeffrey x (Jeffrey x Coulter)	14.6	53	62*	--	--	--	--	--

1/ Test c at 8 days.

2/ Six 10-beetle replicates per treatment.

3/ * = significantly greater than control at 95 percent confidence level; ** = significantly smaller.

The supernatant liquid of ponderosa pine caused mortality which was very similar to that caused by fresh resin. Two different ponderosa pines which were tested caused about the same amount of mortality. Apparently D. monticolae is unaffected by ponderosa pine resin vapors under certain conditions; but under other conditions it is deleteriously affected. This could be due to changes in either the beetle or the resin vapor or in both.

Resin of Coulter and lodgepole pines, which both are hosts of D. monticolae, caused no significant increase in mortality.

Resin of the nonhosts Monterey pine and knobcone x Monterey hybrid caused a nonsignificant increase in mortality, although last year both resins caused a significant increase. The resin-caused mortality, about 60 percent, was almost the same in both years; but in the 1961 tests natural mortality was 45 percent, or almost double the natural mortality in 1960. Knobcone pine resin vapor caused a significant

increase in mortality in two tests, but not in a third. The general level of mortality caused by the resin vapor of these two nonhost pines and their hybrid was somewhat higher than that caused by host pines such as Coulter, lodgepole, and ponderosa.

Resins of the Jeffrey x Coulter and the Jeffrey x (Jeffrey x Coulter) hybrids were used in two tests. The Jeffrey x Coulter caused no significant increase in mortality; in fact, in one test it caused a significant decrease. The backcross hybrid caused a significant increase in one test. In all tests a partial paralysis of the beetles was noted which was quite similar to that noted for the Jeffrey x ponderosa hybrid resin vapor.

Feeding Behavior Tests

Tests in 1960 showed that saturated resin vapors of the Jeffrey x ponderosa hybrid had no toxic effect on D. monticolae as measured by the vapor toxicity technique. However, reduced activity of the beetles while in the presence of the vapors was observed, which suggested a nonlethal effect. In order to determine whether this effect actually occurs, extensive testing was carried out using the Jeffrey x ponderosa hybrid as well as another nonhost x host hybrid, Jeffrey x Coulter (table 2). The two hybrids were used in the same test only once; and in this case the 57 mg. of frass that the beetle produced in the presence of Jeffrey x Coulter resin vapor was significantly less than the 122 mg. produced in the presence of Jeffrey x ponderosa resin vapor. In most tests, the beetle produced from 100 to 120 mg. of frass in the presence of either hybrid resin vapor, and in all cases the amount was significantly lower than in the control.

Two series of tests were made with different treatment times, using resin vapor of ponderosa pine and the Jeffrey x ponderosa hybrid. In one series, treatments of 2, 4, 6, and 8 days were used and in the other, the times were 5 and 10 days. These tests showed that the amount of frass produced in the presence of the hybrid resin vapor was significantly reduced at all treatment times. Thus, it would appear that the effect of the resin vapor was about the same throughout the course of the test. Mortality did not increase in any of these feeding tests; and there was no reduction in the number of beetles which attempted to feed.

Results of feeding tests with D. monticolae in the presence of vapors of ponderosa pine resin consistently showed almost no reduction in frass produced throughout the course of the season. The resin vapor caused no significant reduction in frass production in seven out of eight tests. The average weight of frass in common tests for the control and ponderosa pine was respectively 265 and 256 mg. per 10-beetle replicate. In the one test where there was

Table 2.--Frass per replicate in feeding tests with *D. monticolae*, using vapors of indicated resins at 76° ± 3° F. for varying periods of time

Resin	Vapor saturation: average	Test[1] -- Days of treatment: a 5	b 6	c 5	d 5	e 2	e 4	e 6	e 8	f 5	f 10	g 5	h 5	i 5	j 5
	Mg.	Milligrams[2]													
Control	0.0	231	251	263	233	--	--	--	--	297	273	--	209	278	323
Ponderosa	7.7	255	--	309	242	186	186	288	271	254	267	224	202	243	272*
Coulter	6.5	254	236	--	--	--	--	--	--	--	--	--	--	--	--
Jeffrey	23.2	11*	--	--	--	--	--	--	--	--	--	--	--	--	--
Jeffrey x ponderosa	11.1	139*	--	--	122*	81*	120*	137*	154*	106*	162*	90*	--	--	--
Jeffrey x Coulter	13.0	--	113*	130*	57*	--	--	--	--	--	--	--	--	--	--
Ponderosa distillate	8.4	--	--	--	--	--	--	--	--	--	--	205	--	--	--
Sugar	4.7	--	--	265	--	--	--	--	--	--	--	--	--	--	--
Knobcone	10.1	--	--	--	--	--	--	--	--	--	--	--	195	282	277*
Monterey	10.2	--	--	--	--	--	--	--	--	--	--	--	155	--	249*
Knobcone x Monterey	10.5	--	--	--	--	--	--	--	--	--	--	--	190	211	267*

1/ Number of 10-beetle replicates per treatment as follows: 3 = a; 4 = b, c, e, g, h; 5 = f, j; 6 = i; 7 = d.

2/ * = significantly smaller than control at 95 percent confidence level.

a significant reduction, the untreated control had an unusually large amount of frass (323 mg.). In tests common for the control and Coulter pine, the average quantity of frass produced was 242 and 244 mg., respectively. Two other host resin materials, sugar pine resin and ponderosa pine resin distillate, caused no significant reduction in frass production.

A significant reduction in frass occurred in feeding tests with the resin vapor of the nonhost Jeffrey pine. This, however, was due largely to the high beetle mortality; i.e., the beetles did not live long enough to do much feeding.

Three tests were made with resin vapors of knobcone and Monterey pines and the knobcone x Monterey hybrid along with ponderosa pine and suitable controls. The hybrid and Monterey pines had caused a significant increase in mortality in the 1960 vapor toxicity tests, though no unusual effect on the beetle's activity was noticed. None of the resins in these three feeding tests reduced the amount of frass produced significantly although Monterey resin vapor caused a sizable reduction. The hybrid caused less of a reduction than Monterey while knobcone caused the least reduction. The average amount of frass per 10-beetle replicate was 275, 257, 224, and 207 mg., respectively, for the control, knobcone, knobcone x Monterey, and Monterey pines.

A proper question is why do these resins cause a significant increase in mortality in the vapor toxicity tests, but no significant reduction in the feeding tests. The presence of the great absorptive surface of the particles of frass in the feeding tests is the most logical answer. As the data in tables 1 and 2 show, there was an apparent increase in vapor saturation in all feeding tests compared to the toxicity tests. This increase probably was caused by the withdrawal of the vapor from the atmosphere by the particles of frass. As vapor was removed, more would evolve from the source and thus cause an apparent increase in the amount required for saturation. The actual result may have been a reduction in vapor saturation since the tremendous absorptive surface and volume of the frass may have removed the vapor fast enough to prevent the chamber from being saturated. It is entirely possible for the frass to differentially absorb the various vapor constituents, introducing another uncertainty in this already complicated situation. Until more facts are available about how the presence of frass in the chamber affects resin vapors, results obtained with this procedure must be viewed with reservation.

DENDROCTONUS BREVICOMIS

Vapor Toxicity Tests

Seven vapor toxicity tests were made with *D. brevicomis* and various pine resins or resin derivatives using the standard procedure, with a 5- or 6-day period of exposure (table 3). Ponderosa pine resin and an untreated control were included in each test.

Table 3.--Mortality of *D. brevicomis* in vapor toxicity tests using saturated vapors of indicated resins for 5 days[1/] at 75° ± 2° F.

Resin	Vapor saturation average	Test[2/] a	b	c	d	e	f	g
	Mg.	Percent[3/]						
Control	0.0	14	14	35	49	28	25	10
Ponderosa #1	3.2	25	42*	61*	74*	39	35	17
Ponderosa #2	2.6	--	--	53*	--	--	--	--
Coulter	2.1	--	--	--	63	38	--	--
Knobcone	5.5	25	--	--	--	56*	--	--
Lodgepole	2.3	31	--	--	--	53*	--	--
Jeffrey x (Jeffrey x Coulter)	13.5	44*	--	--	--	--	--	--
Ponderosa supernatant	3.1	--	40*	46	--	--	47	--
Ponderosa distillate #1	9.4	88*	--	--	--	--	--	--
#2	5.1	89*	--	--	--	--	--	--
#3	4.5	--	--	--	--	--	--	53*
#4	7.7	--	--	--	--	--	--	60*
#5	7.0	--	--	--	--	--	--	4/ 72*

1/ d was for 6 days.

2/ Six 12-beetle replicates for a, b, c, d, e; five for f, g.

3/ * = significantly greater than control at 95 percent confidence level.

4/ Significantly greater than distillate #3.

Variable results were achieved with the ponderosa resin for reasons which cannot be explained adequately at this time. In three of the tests, those conducted toward the latter part of the summer, the resin caused no significant increase in beetle mortality. However, a

significant increase was registered in tests conducted in the early part of the summer. A wide range in natural mortality occurred in the controls, and there was a shift in vapor saturation of resin; but neither variable shifted in a regular pattern. Vapor saturation greater than 3.0 mg. was associated with all but one of the tests where a significant increase in mortality occurred. It remains to be answered whether shifts in vapor saturation and rate of natural mortality are important factors in the reaction of the beetles to ponderosa pine resin vapor.

In the one test conducted with resins of two different ponderosa pines there was no difference in beetle mortality. One caused 61 percent kill and the other 53 percent. Coulter pine, the other host of the beetle, caused no significant increase in mortality. The effects of ponderosa pine resin and its supernatant liquid were quite similar. The mortality caused by resin and the supernatant liquid in the three tests where these materials were used differed by only 2, 15, and 12 percent. The effect of ponderosa resin and its liquid distillate was quite dissimilar. In one of the two tests in which both materials were used, mortality with resin vapor was 25 percent; with two different distillates it was 88 percent and 89 percent. In the other tests, resin vapor caused 17 percent mortality, while three distillates, obtained at three different times, caused 53, 60, and 72 percent mortality. The difference between the lowest and highest percent mortality with the distillates was significant. The distillate causing 53 percent mortality was obtained on July 8, while that causing the 72 percent mortality was obtained on September 13. This suggests seasonal changes in ponderosa pine resin which affect its toxicity to beetles.

Two nonhost resins, knobcone and lodgepole, caused a significant increase in mortality in one test but failed to do so in another. The Jeffrey x (Jeffrey x Coulter) pine resin vapor caused a significant increase in mortality in the one test conducted.

Feeding Behavior Tests

The feeding reaction of *D. brevicomis* in the presence of saturated resin vapors was determined in five tests (table 4). The hybrid pines, Jeffrey x ponderosa and Jeffrey x Coulter, produced highly significant reductions in feeding. Resin vapors of these pines have not shown consistently a lethal effect on the beetles in vapor toxicity tests, although they have shown a tendency to inhibit the activity of the beetles. Thus, the reduction in feeding was not due to increased mortality; instead it appears to be due to a reduction in the amount of feeding the beetles accomplish.

Table 4.--Frass per replicate in feeding tests with *D. brevicomis*, using vapors of indicated resins at 76° ± 3° F. for 5 days

Resin	Vapor saturation average	Test[1/]				
		a	b	c	d	e
	Mg.	Milligrams[2/]				
Control	0.0	35	29	46	37	38
Ponderosa	8.0	37	42	38	54	38
Coulter	6.1	34	--	--	--	--
Jeffrey	19.5	3/ 0.5*	--	--	--	--
Jeffrey x ponderosa	12.7	2*	--	3*	--	--
Jeffrey x Coulter	12.9	--	4*	2*	--	--
Sugar	4.6	--	31	--	65**	--
Western white	5.0	--	--	--	46	--
Monterey	11.0	--	--	--	--	48
Knobcone	11.5	--	--	56	--	40
Knobcone x Monterey	12.0	--	--	34	--	44

1/ Number of 10-beetle replicates per treatment as follows: a = 3, b = 4, c = 6, d = 6, e = 5.

2/ * = significantly smaller than control at 95 percent confidence level; ** = significantly larger.

3/ Significantly smaller than Jeffrey x ponderosa.

Resins of host pines, ponderosa and Coulter, caused no significant reduction in feeding. In the ponderosa pine tests, the 42 mg. of frass produced per replicate was very similar to the 37 mg. in the control. Although Coulter pine resin was used in only one test, the results were about the same; e.g., 34 mg. of frass per replicate.

The two soft pines, sugar and western white, neither of which is a host of *D. brevicomis*, caused no significant reduction in feeding in these tests. Previous vapor toxicity studies have shown that soft pine resins do not have a lethal effect on this beetle, but it was anticipated that the feeding technique experiments might reveal a sublethal effect. This was not the case, however; the feeding behavior tests do not enable us to clarify the resin-beetle relationship of soft pines.

Quite surprisingly the two closed-cone pines and their hybrid (knobcone, Monterey, and knobcone x Monterey) caused no reduction in frass production. These pines are nonhosts of the test beetle; and if the resin vapor had shown a sublethal effect it might have helped to explain why. None of these three resin vapors caused a significant increase in mortality during the feeding tests. The adsorption of resin vapors by the frass and bark, as more fully discussed in the section on D. monticolae, seems to be the most logical explanation for the lack of a significant reduction in frass with these resin vapors.

Jeffrey pine, as expected, caused a highly significant reduction in feeding. This is largely attributed to the lethal effect of the resin vapor which caused 100 percent mortality in only 3 days.

DENDROCTONUS JEFFREYI

Only four tests were conducted with D. jeffreyi because of the poor emergence of beetles; and the results may be questionable because the beetles' behavior seemed atypical. In vapor toxicity tests the mortality caused by Jeffrey pine resin vapor was always significantly greater than the control, and in one test it was even significantly greater than that caused by ponderosa resin vapor. Both the uncertain quality of the beetle and the use of ponderosa pine bark, a nonhost material, in the feeding tests seem to invalidate the results of the feeding tests.

RESIN PROPERTIES

Considerable data were obtained on the vapor saturation and percent volatility of resin and resin derivatives from this year's tests, adding to that obtained previously. Vapor saturation is defined as the loss of weight of the resin sample in a 150 cc. airtight chamber at 73° ± 2° F. Percent volatility was calculated on the basis of the original weight of the resin sample and the total loss of weight at the end of a given temperature-time treatment. Vapor saturation information was obtained as part of the toxicity and feeding tests, while data on volatility were obtained subsequent to the tests by conducting a series of temperature-time treatments. An electric oven was used for temperatures greater than room temperature.

The vapor saturation data, listed by species of resin and type of test for 1960 and 1961, are summarized in table 5. There are two striking features of these data: (a) Vapor saturation in the vapor toxicity tests is markedly lower than in feeding behavior tests. This is attributed to the adsorptive area of the bark and frass particles in the feeding behavior tests, which are believed to have removed the vapor from the atmosphere, causing continued vaporization and weight loss of the resin; (b) There were some differences in the saturation for the 2 years for most species of resin; both experi-

mental error and temperature variation are suspected of being responsible for the minor differences. The temperature for the feeding tests was about 2° F. higher than for the vapor toxicity tests. The change was most apparent for two resins in particular, ponderosa and Jeffrey pines. Vapor saturation for ponderosa pine went from 2.6 mg. in 1960 to 3.5 mg. in 1961, an increase of about 35 percent; the change for Jeffrey pine was from 19.7 to 28.6 mg., an increase of about 45 percent. These increases, if they are real, may be associated with the increased toxicity of the resins to the beetles which showed up in this year's tests.

Table 5.--Vapor saturation of pine resins used in vapor toxicity and feeding behavior tests

Pine	1961 Feeding tests	1961 Vapor toxicity	1960 Vapor toxicity
	Milligrams		
Coulter	6.6	2.2	2.1
Sugar	4.6		2.3
Lodgepole		2.2	
Ponderosa	7.7	3.5	2.6
Western white	5.0		4.2
Monterey	9.6	4.6	5.4
Knobcone	9.8	5.8	4.6
Knobcone x Monterey	10.9	5.0	6.1
Jeffrey x ponderosa (1-6)	11.2		8.3
Jeffrey x Coulter	12.7	11.6	9.5
Jeffrey x (Jeffrey x Coulter)		14.6	14.3
Jeffrey	21.3	28.6	19.7

Percent volatility data at room temperature were, likewise, compiled by species for 1960 and 1961 (table 6). These show a high degree of consistency in volatility for the 2 years.

The percent volatility at increased temperatures is given in table 7. The 2-year data for volatility at the end of the 100° C. treatment are compared in the last two columns. There was a change of less than 3 percent for all resins except knobcone. However, the 1960 knobcone volatility is based on only 3 samples and, therefore, poor sampling may be the reason for the 5 percent difference.

Table 6.--Percent volatility of resins of pine species and hybrids, used in vapor toxicity and feeding behavior tests, at 75° ± 3° F. for 5-7 days

Pine resin	Year 1960	Year 1961
	Percent	
Monterey	25.62	27.05
Knobcone x Monterey	25.11	24.39
Knobcone	22.73	22.51
Ponderosa	19.29	20.66
Lodgepole		14.04
Coulter	8.08	9.42
Jeffrey x ponderosa	10.01	11.41
Jeffrey x Coulter	5.27	7.35
Jeffrey x (Jeffrey x Coulter)	7.43	7.53
Jeffrey	11.75	12.62
Sugar	2.12	3.66
Western white	3.88	4.66

Table 7.--Percent volatility of pine resins used in vapor toxicity and feeding behavior tests, at designated temperatures

Pine	50° C. 72 hours	60° C. 48 hours	80° C. 48 hours	100° C. 48 hours 1961[1]	100° C. 48 hours 1960
	Percent				
Monterey	28.52	28.97	30.19	34.55	33.85
Knobcone x Monterey	25.39	25.95	27.91	32.71	30.56
Knobcone	22.07	22.44	24.31	30.01	25.13
Ponderosa	21.93	22.69	24.72	26.17	25.85
Lodgepole	13.70	15.11	18.96	20.96	
Coulter	10.02	13.25	17.92	19.52	17.57
Jeffrey x ponderosa	12.35	13.27	15.84	17.67	17.08
Jeffrey x Coulter	7.63	8.14	14.55	16.28	15.31
Jeffrey x (Jeffrey x Coulter)	6.59	6.41	7.60	10.61	11.53
Jeffrey					18.72
Sugar					17.64
Western white					19.84

1/ With an additional 48 hours at this temperature there was usually less than 0.5 increase in the percent volatility.